Exhibit A

PATENT
Docket No. 7823/5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of Matthew B. Wheeler	Group Art Unit: 1819
Serial No.: 08/410,539	Examiner: Bruce R. Campell
Filed: March 24, 1995	
For: TRANSGENIC UNGULATE METHODS AND COMPOSITIONS	

DECLARATION OF MATTHEW B. WHEELER
UNDER 37 C.F.R. § 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

Dear Sir:

I, Matthew B. Wheeler, hereby declare that:

1. I am the inventor of the patent application captioned above ("application").

2. My relevant experience and background in the field of livestock breeding and tissue cultures are as follows:

I obtained a Ph.D. in Physiology and Biophysics/Cell and Molecular Biology, Colorado State University. After obtaining the Ph.D., I was a Post-Doctoral Fellow at the University of Virginia School of Medicine, and a Research Associate at the University of Wisconsin.

Presently, I am an Associate Professor, Department of Animal Science, University of Illinois at Urbana-Champaign.

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I have published more than 40 book chapters and journal articles on aspects of reproduction in animals.

3. I have read the Office Action of March 17, 1997.

4. Contrary to the examiner's conclusion, I believe the specification is enabling for ungulates.

5. As demonstrated in the following paragraphs, I have prepared ES cells from sheep without undue experimentation, following the instructions in the pending specification.

6. Embryonic stem cells were isolated from sheep blastocysts and maintained in culture. Mature (>3 yr) Dorset ewes served as embryo donors to provide embryonic stem (ES) cells. Donors were checked for estrus twice daily and naturally inseminated by a crossbred Texel X Suffolk ram following the onset of behavioral estrus. Hatched blastocysts were flushed from the uterus of the donors 7-8 d after the first day of estrus (d=0) with Dulbecco's phosphate buffered saline (D-PBS; 45) which contained 5% fetal calf serum (FCS, Sigma # F-2442, Sigma Chemical Co., St. Louis, MO). Embryos were washed three times in either D-PBS or BECM and cultured individually on mitomycin C-inactivated mouse embryonic fibroblasts (STO)

monolayers with 0.5 ml of conditioned stem cell medium (CSCM). This system is the same that we have employed to isolated porcine ES cells.[1]

7. The inner cell mass (ICM) of the cultured sheep embryo was evident during the first 1-14 days of culture. After the ICM emerged, usually 7-9 days in culture, the whole embryonic cell colony was partially disaggregated with buffered trypsin/EDTA solution for 1-2 min at 37-39 °C. The trypsin/EDTA solution was diluted 1:1 with CSCM, triturated and the partially disaggregated cells were re-seeded onto new mitomycin C-inactivated STO feeder layers. For all subsequent passages the cells were plated onto fresh feeder layers with BRL conditioned stem cell media (CSCM). Cells were subcultured as necessary onto increasingly larger culture dishes.

8. Initial attachment of the hatched sheep blastocyst to the feeder layer or culture vessel was similar to the pig. In the sheep, as in the pig, the hatched blastocyst attaches and plates down in a large clump and then begins to spread out as if it were melting. Consequently, the ICM was associated with trophoblast cells, and its configuration resembled a fried egg in appearance. This phenomenon makes it difficult initially (first several days, 1-5) to

[1] Conditioned stem cell medium is comprised of 40% Dulbecco's Modified Eagle's Medium (DMEM; containing L glutamine, 4500 mg glucose/L; Sigma Hybrimax #D6655, Sigma Chemical Co., St. Louis, MO with the following supplements: 20% FCS, 0.1 mM 2-mercaptoethanol, 50 IU penicillin/L, 50 Fg streptomycin/L, 10 mM/L MEM non-essential amino acids (Sigma #M7145, Sigma Chemical Co., St. Louis, MO), nucleosides (.03 mM adenosine, .03 mM guanosine, .03 mM cytidine, .03 mM uridine and .01 mM thymidine) and 60% Buffalo Rat Liver cell conditioned medium (BRL-CM) containing a total of 20% FCS, and the outlined supplements.

pluck the ICM alone, and as a result, depending on the plated configuration of the individual embryo, the ICM may be plucked or the entire plated embryo may be trypsinized to dissociate the cells. After discrete multilayered clumps or colonies of ES-cells are visible, then "plucking" was done to isolate these cells from contaminating trophoblast and/or other differentiated cell types. This results in isolation of cells with the proper morphology, that is, as described in the pending application.

9. Serial subculture was performed at intervals that are a function of culture growth rate, but usually at intervals from 2-3 days (range 2-14 days). This is faster than in the pig. Subculturing was continued until a stable culture with morphological features and growth parameters characteristic of an embryonic stem cell culture (as described below) was established. We determined that an ES culture from sheep was established because the morphology and growth were consistent with those for swine.

10. The ES cells derived from sheep blastocysts grow in distinct raised colonies as opposed to monolayers. The colony morphology is similar to that of the mouse ES D3 cells of Doestchman (Development 102, 471-478.). As in the pig, the diameter of the colonies ranged from 0.08 to 1.5 mm four days after plating as a single cell suspension. Sheep ES cells are small (8-15 microns diameter), rounded and dark, yet translucent. The nucleus, contains several prominent nucleoli and makes up ~80% of the cell volume. The surface of the colony is irregular as is the surface of individual cells. The average doubling time of the sheep ES cells is 18-48 hr after passages 5-7 and is relative constant thereafter.

11. We noticed some differences between swine and sheep ES cell morphology and growth, but these differences do not interfere with recognition of the cells as "ES cells." The colonies are slightly smaller than those seen in the pig and they grow faster than the pig cells. The individual diameter of the cells may be slightly larger 8-15μm vs. 8-12μm for the pig! The culture require subculture every 2-3 days (2-7 days is the range). We have also noted that greater than 95% of the sheep embryos we put into this culture system have produced ES cell lines which have been cultured in vitro for at least 6 passages. This percentage is greater than that in the pig which is currently about 80%.

12. We currently have one vial of a pass 7 sheep ES cell line (S27) frozen containing 3 X 10^6 cells. These cells were frozen on December 7, 1994.

* * * * * *

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

September 15, 1997
Date

Matthew B. Wheeler

Matthew B. Wheeler